

WHAT WE CLAIM IS:

1. An expression cassette, operable in a recombinant host, comprising a heterologous DNA coding sequence encoding a protein, which is functional, alone or in cooperation with one or more additional proteins, of catalyzing an oxidation step in the biological pathway for conversion of cholesterol into hydrocortisone, which step is selected from the group consisting of:

the conversion of cholesterol to pregnenolone;

the conversion of pregnenolone to progesterone;

the conversion of progesterone to 17 $\alpha$ -hydroxy-progesterone;

the conversion of 17 $\alpha$ -hydroxyprogesterone to cortexolone;

the conversion of cortexolone to hydrocortisone, and the corresponding control sequences effective in said host.

2. An expression cassette of Claim 1, wherein the heterologous DNA coding sequence encodes at least two proteins which are functional of catalyzing, alone or in cooperation with one or more additional proteins, at least two oxidation steps of the group of Claim 1.

3. An expression cassette of Claim 1, wherein it contains at least one additional heterologous DNA with its own effective control sequences, encoding a protein, which is functional, alone or in cooperation with one or more additional proteins, of catalyzing an oxidation step of the group of Claim 1.
4. An expression cassette of Claim 1 wherein the protein is selected from the group consisting of side-chain cleaving enzyme ( $P_{450}SCC$ ); adrenodoxin (ADX); adrenodoxin reductase (ADR);  $3\beta$ -hydroxysteroid dehydrogenase/isomerase ( $3\beta$ -HSD); steroid- $17\alpha$ -hydroxylase ( $P_{450}17\alpha$ ); NADPH cytochrome  $P_{450}$  reductase (RED); steroid- $21$ -hydroxylase ( $P_{450}C21$ ), and steroid- $11\beta$ -hydroxylase ( $P_{450}11\beta$ ).
5. An expression cassette of Claim 4, wherein the heterologous DNA coding sequences originate from bovine species.
6. An expression cassette of Claim 4 wherein the heterologous DNA encodes at least one additional protein from the group of Claim 4.
7. An expression cassette of Claim 4 wherein it contains at least one additional heterologous DNA with its own effective control sequences encoding a protein from the group of Claim 3.

8. An expression cassette of Claim 6 wherein the heterologous DNA encodes bovine  $P_{450}SCC$  and bovine ADX.
9. An expression cassette of Claim 5 wherein the heterologous DNA encodes the enzyme  $P_{450}SCC$  and that the expression cassette is denoted as  $pGBSCC-n$  where  $n$  is an integer from 1 to 17.
10. An expression cassette of Claim 5 wherein the heterologous DNA encodes the enzyme  $P_{450}17\alpha$  and the expression cassette is denoted as  $pGB17\alpha-n$  where  $n$  is an integer from 1 to 5.
11. An expression cassette of Claim 5 wherein the heterologous DNA encodes the enzyme  $P_{450}C21$  and the expression cassette is denoted as  $pGBC21-n$  where  $n$  is an integer from 1 to 9.
12. An expression cassette of Claim 5 wherein the heterologous DNA encodes the enzyme  $P_{450}11\beta$  and the expression cassette is denoted as  $pGB11\beta-n$  where  $n$  is an integer from 1 to 4.
13. A recombinant host cell and progeny thereof comprising cells of micro-organisms, plants or animals and containing an expression cassette with heterologous DNA characterized in that the expression cassette is that of Claim 1.
14. A recombinant host cell and progeny thereof of Claim 13

wherein the host is a micro-organism.

15. A recombinant host cell and progeny thereof of Claim 14 wherein the host is selected from the group consisting of Saccharomyces, Kluyveromyces, Bacillus and Escherichia coli.
16. A recombinant host cell and progeny thereof of Claim 13 and containing at least two expression cassettes as defined in Claim 1.
17. A process for the preparation of an exogenous protein by a recombinant cell comprising culturing the recombinant cell in a nutrient medium under conditions enabling the protein to be formed and accumulated in the culture, wherein the recombinant cell is a recombinant host cell as defined in Claim 13.
18. A process for the preparation of a mixture of endogenous proteins by a recombinant cell in a nutrient medium under conditions enabling the enzymes to be formed and accumulated in the culture wherein the recombinant cell is a recombinant host cell as defined in Claim 16.
19. A process for selective biochemical oxidation in vitro comprising incubating the compound to be oxidized in the presence of at least one protein under conditions which

permit said oxidation and the accumulation of the oxidized compound in the culture liquid, followed by recovering the oxidized compound, characterized in that the protein or proteins have been produced by the process of Claims 17.

20. In a process for oxidizing a selected compound comprising culturing recombinant cells in the presence of said compound under conditions wherein the desired oxidation occurs and the oxidized compound accumulates in the culture liquid and recovering the oxidized compound, the improvement comprising using a recombinant host cell of Claim 13.
21. A process of Claim 20, wherein the oxidation is a step selected from the group consisting of: cleaving the side-chain of a sterol compound to pregnenolone; the conversion of pregnenolone to progesterone; the conversion of progesterone to 17 $\alpha$ -hydroxyprogesterone; the conversion of 17 $\alpha$ -hydroxyprogesterone to cortexolone and the conversion of cortexolone to hydrocortisone.
22. The process of Claim 21 wherein the oxidation is cleaving the side-chain of a sterol compound resulting in pregnenolone.
23. The process of Claim 21 wherein the oxidation is the 17 $\alpha$ -hydroxylation of progesterone.

24. The process of Claim 21 wherein at least two oxidations from said group are carried out on the same substrate molecule in one step.

25. A recombinant host cell and progeny thereof comprising cells of micro-organisms, plants or animals containing, besides proper control sequences, heterologous DNA coding for proteins either from the group comprising proteins which are functional, alone or in cooperation with one or more additional proteins, of catalyzing an oxidation step in the biological pathway for conversion of cholesterol into hydrocortisone, which step is selected from the group consisting of: the conversion of cholesterol to pregnenolone;

the conversion of pregnenolone to progesterone;

the conversion of progesterone to 17 $\alpha$ -hydroxy-progesterone;

the conversion of 17 $\alpha$ -hydroxyprogesterone to cortexolone; and the conversion of cortexolone to hydrocortisone

or from the group comprising the natural proteins of the biological pathway for conversion of cholesterol into hydrocortisone being selected from the group consisting of: side-chain cleaving enzyme (P<sub>450</sub>SCC); adrenodoxin (ADX); adrenodoxin reductase (ADR); 3 $\beta$ -hydroxysteroid dehydrogenase/isomerase (3 $\beta$ -HSD); steroid-17 $\alpha$ -hydroxylase

(P<sub>450</sub>17 $\alpha$ ); NADPH cytochrome P<sub>450</sub> reductase (RED); steroid-21-hydroxylase (P<sub>450</sub>C21), and steroid-11 $\beta$ -hydroxylase (P<sub>450</sub>11 $\beta$ ), characterized by heterologous DNA encoding at least two expressible proteins which catalyze at least two separate oxidation steps.

26. A recombinant host cell of Claim 25 wherein the heterologous DNA coding sequences originate from bovine species.

27. A recombinant host cell of Claim 25 wherein the heterologous DNA encodes at least the P<sub>450</sub>17 $\alpha$  and the P<sub>450</sub>C21 proteins.

28. A recombinant host cell and progeny thereof of Claim 25 wherein the host is a micro-organism.

29. A recombinant host cell and progeny thereof of Claim 28 wherein the host is a species selected from the group consisting of Saccharomyces, Kluyveromyces, Bacillus and Escherichia coli.

30. In a process for the preparation of hydrocortisone from sterols, comprising culturing a recombinant cell in a nutrient medium, the improvement comprising a recombinant host containing an expression cassette, operable in a recombinant host, comprising a heterologous DNA coding sequence encoding a protein, which is functional, alone or in cooperation with one or more

additional proteins, of catalyzing at least one oxidation step in the biological pathway for conversion of cholesterol into hydrocortisone, which step is selected from the group consisting of:

the conversion of cholesterol to pregnenolone;  
the conversion of pregnenolone to progesterone;  
the conversion of pregnenolone to 17 $\alpha$ -hydroxyprogesterone;  
the conversion of 17 $\alpha$ -hydroxyprogesterone to cortexolone;  
the conversion of cortexolone to hydrocortisone, and the corresponding control sequences effective in said host.

31. An expression cassette of claim 4, wherein the heterologous DNA encodes the enzyme 3 $\beta$ -HSDH.

32. The process of claim 21, wherein the oxydation is the 3 $\beta$ -hydroxydeshydrogenation/isomerization of pregnenolone.

33. A recombinant host cell of claim 25, wherein the heterologous DNA encodes at least the P450 17 $\alpha$  and the 3 $\beta$ -HSDH proteins.